ENT COOPERATION TRE

Y. (1)	From the INTERNATIONAL BUREAU				
PCT	To:	To:			
NOTIFICATION OF THE RECORDING OF A CHANGE (PCT Rule 92bis.1 and Administrative Instructions, Section 422) Date of mailing (day/month/year)	BERESKIN & PARR 40th floor 40 King Street West Toronto, Ontario M5H 3Y2 CANADA				
13 July 2001 (13.07.01)	L				
Applicant's or agent's file reference 6857-7		IMPORTANT NOT	IFICATION		
International application No. PCT/CA99/01157		nal filing date (day/month/y ecember 1999 (03.12.9			
1. The following indications and a second se	·				
The following indications appeared on record concerning: W the applicant the inventor	the agent	the comm	on representative		
Name and Address		State of Nationality	State of Residence		
BIONICHE INC. 383 Sovereign Road London, Ontario N6M 1A3		CA Telephone No.	CA		
Canada	}	Facsimile No.			
		Teleprinter No.			
2. The International Bureau hereby notifies the applicant that the	ne following	change has been recorded	concerning:		
the person X the name the add		the nationality	the residence		
Name and Address		State of Nationality CA	State of Residence CA		
BIONICHE LIFE SCIENCES INC. 383 Sovereign Road London, Ontario N6M 1A3	-	Telephone No.	<u> </u>		
Canada	-	Facsimile No.			
	ŀ	Teleprinter No.	-		
3. Further observations, if necessary:	<u> </u>				
3. Future observations, in necessary.					
4. A copy of this notification has been sent to:					
X the receiving Office	Γ	the designated Offices	concerned		
the International Searching Authority	Ī	The elected Offices cor	ncerned		
the International Preliminary Examining Authority		other:			
The International Durant - 41800	Authorized	officer	<u></u>		
The International Bureau of WIPO 34, chemin des Colombettes		F. Baechler			
1211 Geneva 20, Switzerland	Talanhons	No.: (41-22) 338.83.38			
Facsimile No.: (41-22) 746 14.35	relebuone.	*O., 17 1 ZZ1 300.00.00			



P ~ ENT COOPERATION TREAT ^

	From the INTERNATIONAL BUREAU
PCT	To:
NOTIFICATION OF ELECTION (PCT Rule 61.2)	Assistant Commissioner for Patents United States Patent and Trademark Office Box PCT Washington, D.C.20231 ETATS-UNIS D'AMERIQUE
Date of mailing (day/month/year)	in its capacity as elected Office
21 August 2000 (21.08.00)	
International application No. PCT/CA99/01157	Applicant's or agent's file reference 6857-7
International filing date (day/month/year)	Priority date (day/month/year)
03 December 1999 (03.12.99)	04 December 1998 (04.12.98)
Applicant	•
PHILLIPS, Nigel, C. et al	
1. The designated Office is hereby notified of its election made X in the demand filed with the International Preliminary 21 June 2000 (Examining Authority on: 21.06.00) national Bureau on:
	Authorized officer

Facsimile No.: (41-22) 740.14.35

The International Bureau of WIPO

34, chemin des Colombettes 1211 Geneva 20, Switzerland Charlotte ENGER

Telephone No.: (41-22) 338.83.38

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau



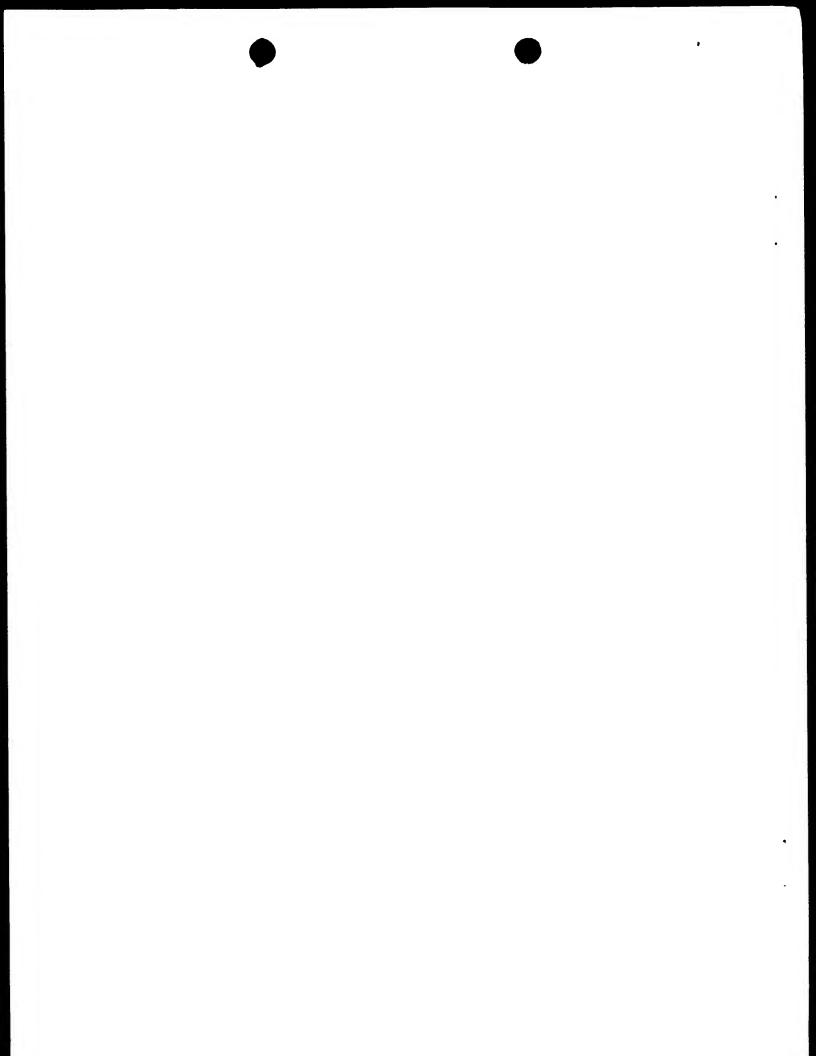
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7: WO 00/33875 (11) International Publication Number: A1 A61K 45/06, A61P 31/00 15 June 2000 (15.06.00) (43) International Publication Date: (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, (21) International Application Number: PCT/CA99/01157 BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, (22) International Filing Date: 3 December 1999 (03.12.99) KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, (30) Priority Data: UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, US 4 December 1998 (04.12.98) 60/111,019 MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, 1 April 1999 (01.04.99) US 60/127,320 BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, (71) Applicant (for all designated States except US): BIONICHE INC. [CA/CA]; 383 Sovereign Road, London, Ontario N6M GN, GW, ML, MR, NE, SN, TD, TG). 1A3 (CA). Published (72) Inventors; and With international search report. (75) Inventors/Applicants (for US only): PHILLIPS, Nigel, C. Before the expiration of the time limit for amending the [CA/CA]; 101 Seigniory Avenue, Point-Claire, Quebec claims and to be republished in the event of the receipt of H9R 1J6 (CA). FILION, Mario, C. [CA/CA]; 2377 St. Zotique, Montreal, Quebec H2G 1K3 (CA). amendments. (74) Agent: BERESKIN & PARR; 40th floor, 40 King Street West, Toronto, Ontario M5H 3Y2 (CA).

(54) Title: CHEMOTHERAPEUTIC COMPOSITION AND METHOD

(57) Abstract

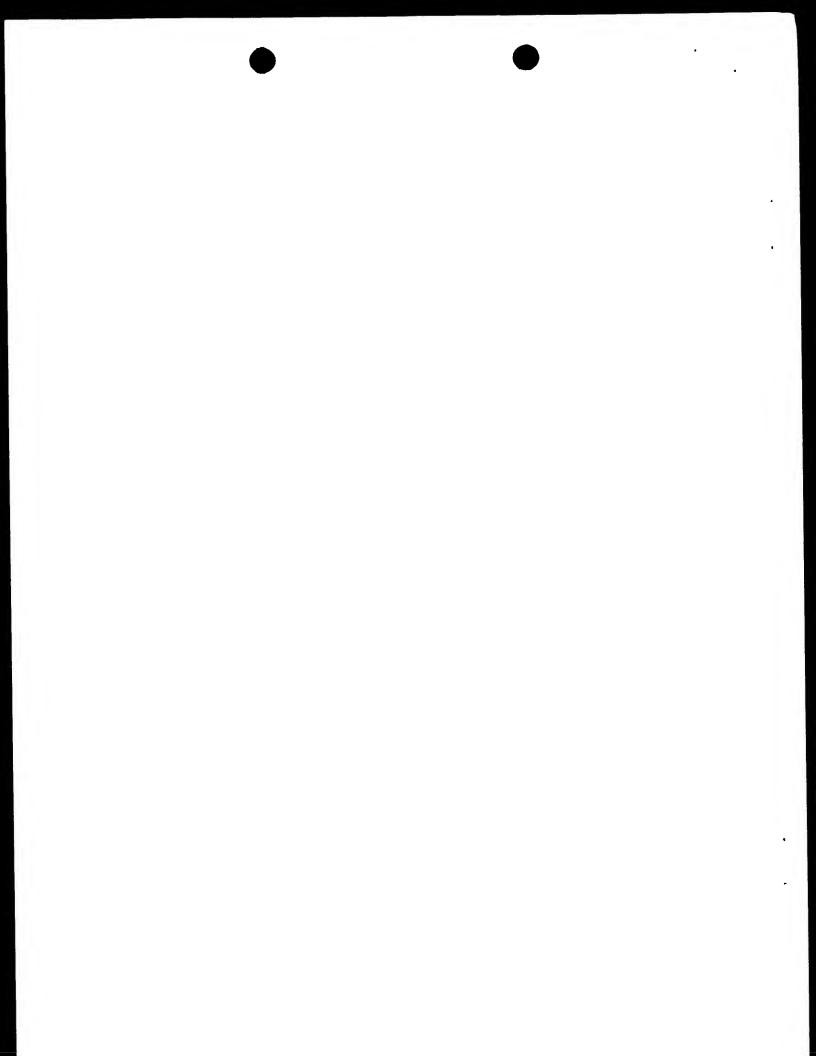
The present invention relates to a composition and method comprising Mycobacterium phlei (M. phlei)-DNA (M-DNA), M-DNA preserved and complexed on M. phlei cell wall (MCC), a chemotherapeutic agent and a pharmaceutically acceptable carrier, wherein the M-DNA and the MCC induce cell cycle arrest in proliferating cancer cells, inhibit proliferation of cancer cells, induce apoptosis in cancer cells and potentiate the antineoplastic effect of the chemotherapeutic agent on cancer cells.



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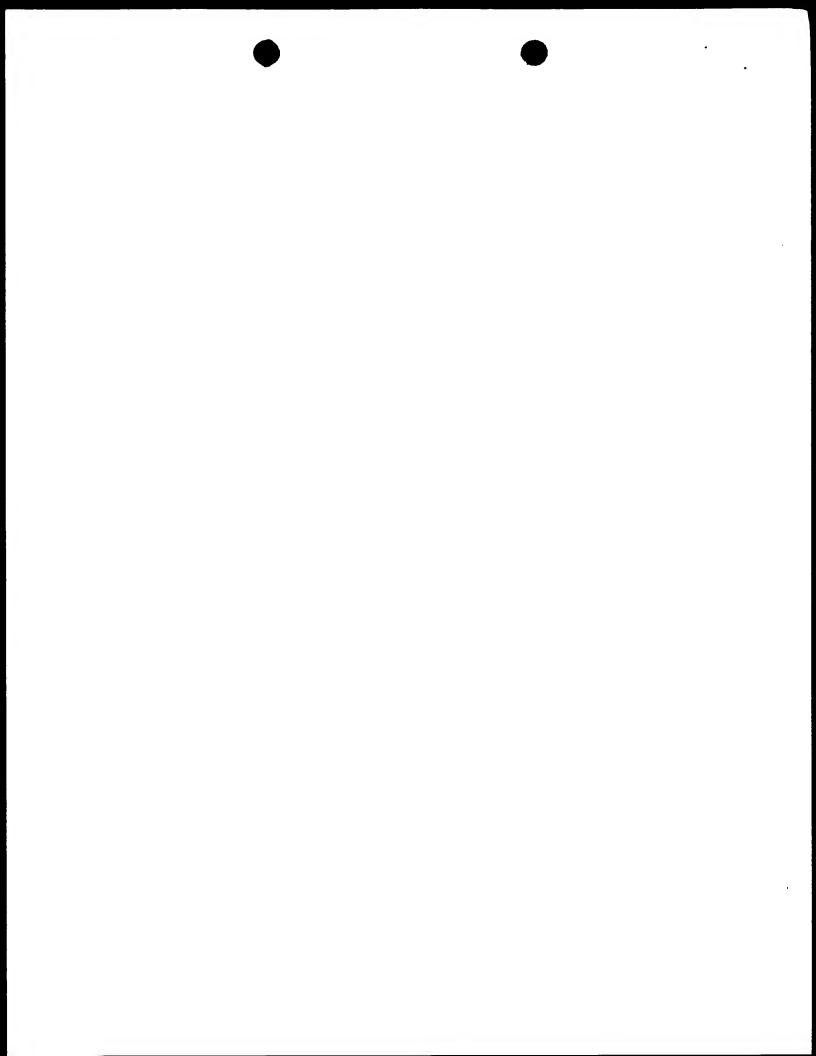






International Ication No PCT/CA 99/01157

A. CLASSII IPC 7	A. CLASSIFICATION OF SUBJECT MATTER IPC 7 A61K45/06 A61P31/00					
According to	nternational Patent Classification (IPC) or to both national classific	ation and IPC				
B. FIELDS	SEARCHED					
Minimum do IPC 7	Minimum documentation searched (classification system followed by classification symbols) IPC 7 A61K					
Documentat	ion searched other than minimum documentation to the extent that	such documents are incl	luded in the fields searched			
	ata base consulted during the international search (name of data ba	use and, where practical	al, search terms used)			
	ENTS CONSIDERED TO BE RELEVANT					
Category °	Citation of document, with indication, where appropriate, of the re	levant passages	Relevant to claim No.			
A	S.YAMAMOTO E.A.: "In vitro augmof natural killer cell activity production of interferon alpha/be-gamma with deoxyribonucleic acid from mycobacterium bovis BcG" JAPANESE JOURNAL OF CANCER RESEAVOL. 79, 1988, pages 866-873, XP page 866 page 872, column 1	and eta and d fraction RCH,				
	ner documents are listed in the continuation of box C.	χ Patent family	/ members are listed in annex.			
"A" docume consid "E" earlier of filing d "L" docume which citation "O" docume other r "P" docume later th	ent which may throw doubts on priority claim(s) or is cited to establish the publication date of another in or other special reason (as specified) sent referring to an oral disclosure, use, exhibition or means and published prior to the international filing date but can the priority date claimed	or priority date an cited to understar invention "X" document of partic cannot be considid involve an invention "Y" document of partic cannot be considid document is comment as each comments, such commin the art. "&" document member	blished after the international filing date not in conflict with the application but not the principle or theory underlying the cular relevance; the claimed invention lered novel or cannot be considered to ive step when the document is taken alone cular relevance; the claimed invention lered to involve an inventive step when the libined with one or more other such docubination being obvious to a person skilled or of the same patent family			
Date of the	actual completion of the international search	Date of mailing of	f the international search report			
1.	2 April 2000	18/04/2	2000			
Name and n	nailing address of the ISA European Patent Office, P.B. 5818 Patentiaan 2 NL – 2280 HV Rijswrik Tel. (+31–70) 340–2040, Tx. 31 651 epo ni, Fax: (+31–70) 340–3016	Authorized officer				

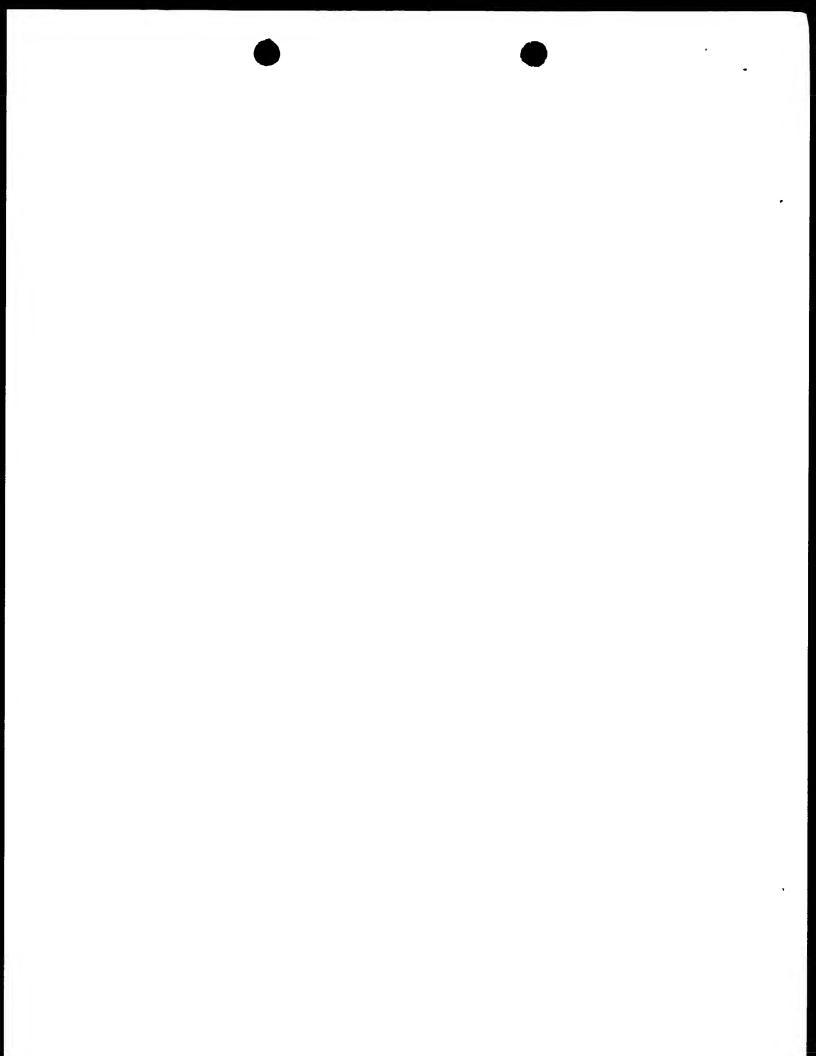






International . cation No PCT/CA 99/01157

		PC1/CA 99/0115/
	ation) DOCUMENTS CONSIDERED TO BE RELEVANT	
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US FILION, M. C. (1) ET AL: "Mycobacterial cell wall-DNA complex induces apoptosis in cancer cells." retrieved from STN XP002133100 abstract å JOURNAL OF PHARMACY AND PHARMACOLOGY, (SEPT., 1998) VOL. 50, NO. SUPPL., PP. 39. MEETING INFO.: 135TH MEETING OF THE BRITISH PHARMACEUTICAL CONFERENCE EASTBOURNE, ENGLAND, UK SEPTEMBER 8-11, 1998,	1,2
Ρ,Χ	W0 99 07383 A (BIONICHE) 18 February 1999 (1999-02-18) claims 1-3,5,6,9-11 page 10, line 29-31 page 11, line 23-28	1-42

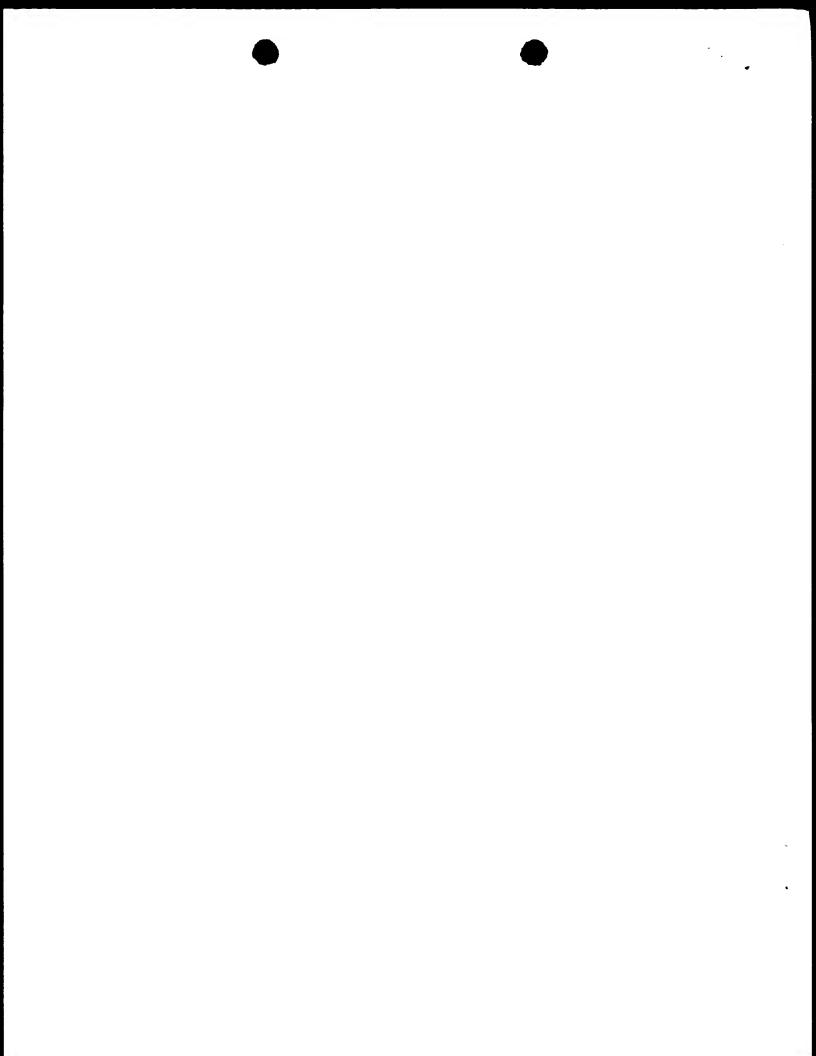




Informa. -- on patent family members

International . cation No PCT/CA 99/01157

Patent document cited in search repor	t	Publication date		Patent family member(s)	Publication date
WO 9907383	Α	18-02-1999	AU	87 23698 A	01-03-1999
			AU	17 46599 A	06-09-1999
			WO	9942113 A	26-08-1999







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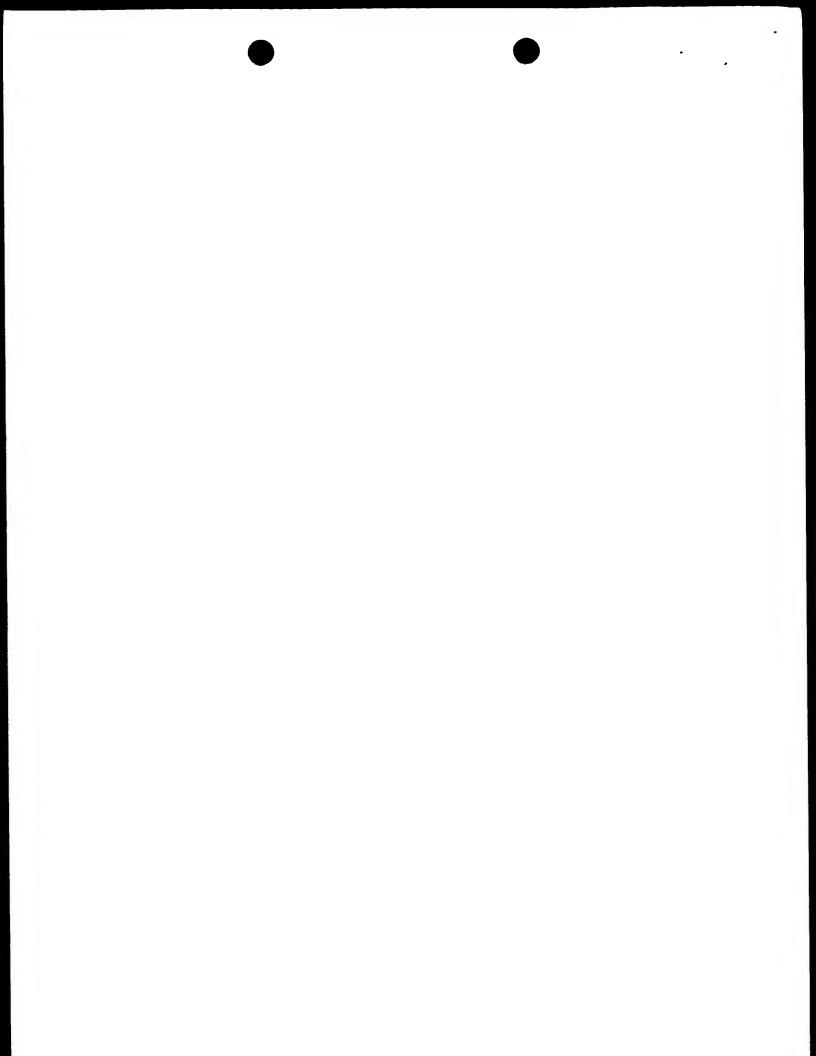
INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

		(1 O1 Article 50 and			15
	or agent's file reference	FOR FURTHER ACTION		ation of Transmittal of International	
6857-00	7	TOTT OTTIER ACTION	Preliminary	Examination Report (Form PCT/IPE	.A/416)
Internation	al application No.	International filing date (day/month	/year)	Priority date (day/month/year)	
PCT/CA	99/01157	03/12/1999		04/12/1998	
Internation A61K45/		or national classification and IPC			
Applicant					
BIONICH	HE(LIFE SCIENCE) IN	C. et al.			
1. This i	nternational preliminary of transmitted to the applic	examination report has been prepared cant according to Article 36.	by this Inte	rnational Preliminary Examining	Authority
2. This f	REPORT consists of a to	tal of 5 sheets, including this cover sh	eet.		
b (s	een amended and are th	panied by ANNEXES, i.e. sheets of the e basis for this report and/or sheets or on 607 of the Administrative Instruction of sheets.	ontaining red	ctifications made before this Auth	have nority
3. This r	eport contains indications	s relating to the following items:			
1	Basis of the report				
II.	Priority				
111	Non-establishment Non-establish	t of opinion with regard to novelty, inve	entive step a	and industrial applicability	
IV	Lack of unity of inv				
V	Reasoned statement citations and expla	ent under Article 35(2) with regard to n nations suporting such statement	ovelty, inver	ntive step or industrial applicabilit	ty;
VI	Certain document	s cited			
VII	Certain defects in t	he international application			
VIII	⊠ Certain observation	ns on the international application			
Date of subr	nission of the demand	Date of co	ompletion of the	nis report	

Date of submission of the demand	Date of completion of this report
21/06/2000	22.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office	Authorized officer
D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Cattell, James Telephone No. +49 89 2399 8469

Telephone No. +49 89 2399 8468

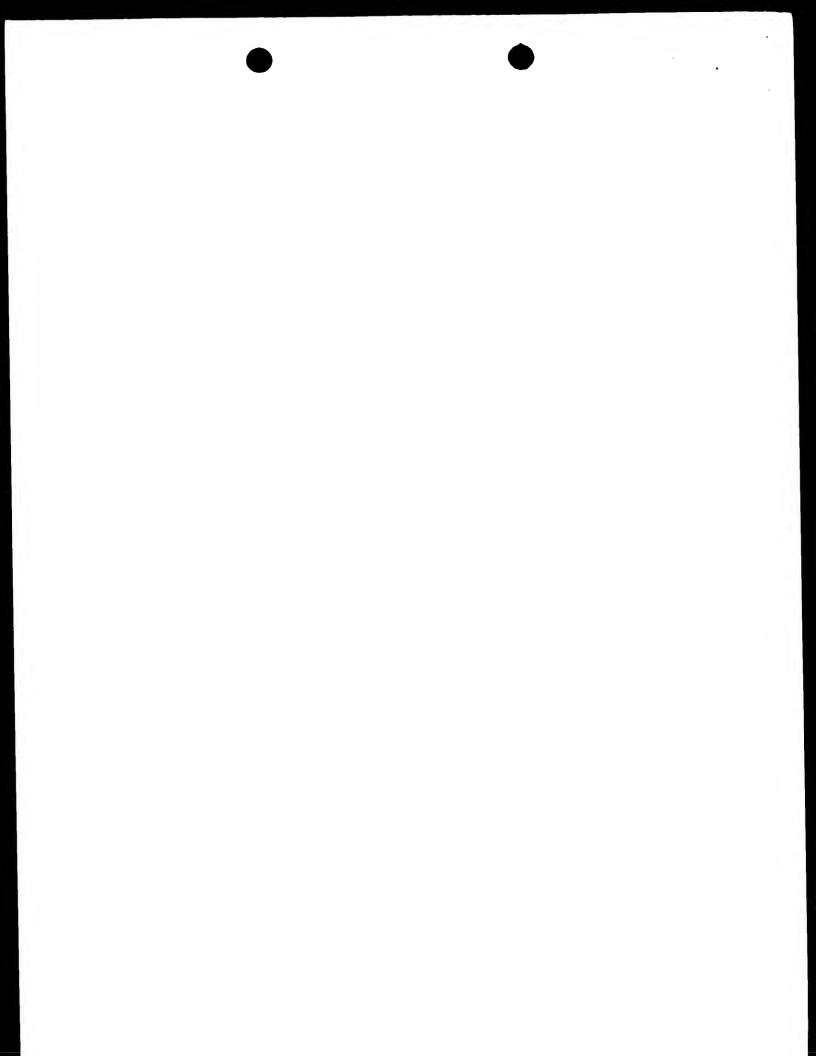


INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA99/01157

I.	Basis	of the	report

1.	res the	This report has been drawn on the basis of (substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).): Description, pages:						
	1-1	4,17,18	as originally filed					
	15,	.16	as received on	15/12/2000	with letter of	15/12/2000		
	Cla	aims, No.:						
	1-1	8	as received on	15/12/2000	with letter of	15/12/2000		
	Dra	awings, sheets:						
	1/9	-9/9	as originally filed					
2.	lanç	guage in which the i	uage, all the elements marked a nternational application was filed available or furnished to this Auth	d, unless othe	rwise indicated under	this item.		
	_					vhich is:		
			ranslation furnished for the purp			der Rule 23.1(b)).		
			blication of the international app	,	. ,,			
	_	55.2 and/or 55.3).	ranslation furnished for the purp	oses of intern	ational preliminary exa	amination (under Rule		
3.	With inte	ith regard to any nucleotide and/or amino acid sequence disclosed in the international application, the ternational preliminary examination was carried out on the basis of the sequence listing:						
		contained in the int	ernational application in written t	form.				
		filed together with t	he international application in co	mputer reada	ible form.			
		furnished subseque	ently to this Authority in written fo	orm.				
		furnished subseque	ently to this Authority in compute	r readable for	m.			
		The statement that the international ap	the subsequently furnished writt plication as filed has been furnis	ten sequence shed.	listing does not go be	yond the disclosure in		
	☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.							

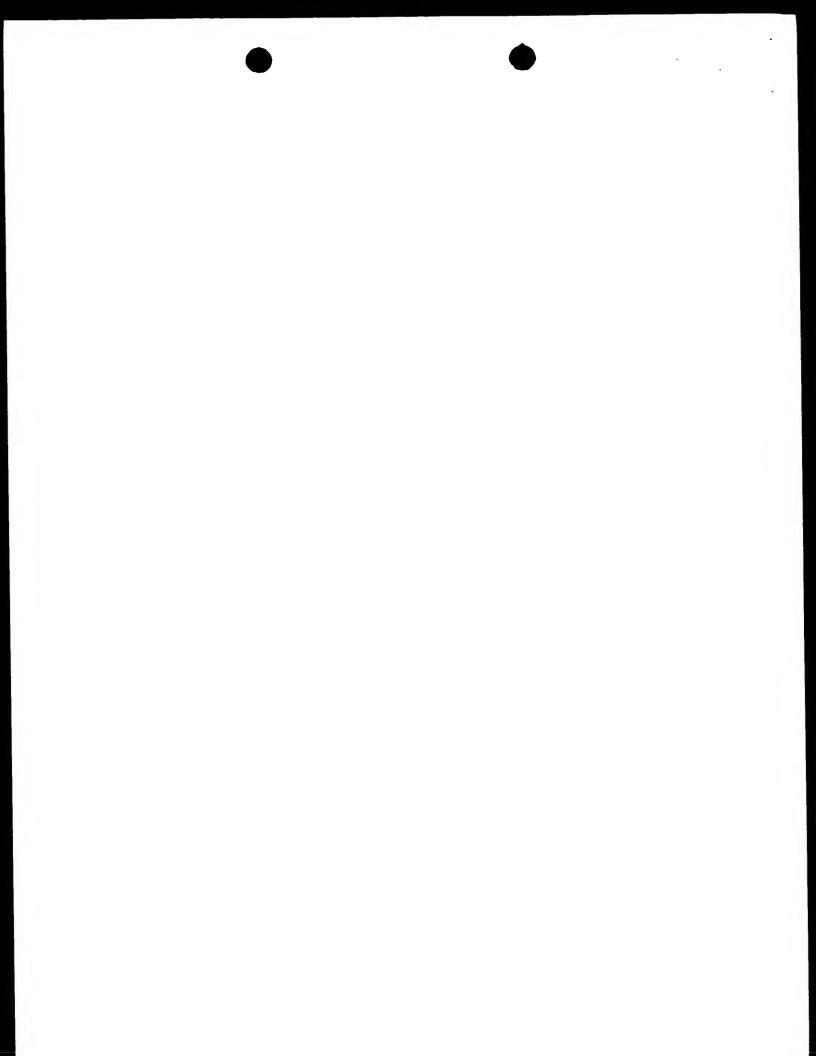


INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA99/01157

4	. Th	e amendments have re	esulted in the cancellation of:
		the description,	pages:
		the claims,	Nos.:
		the drawings,	sheets:
5.		This report has been considered to go bey	established as if (some of) the amendments had not been made, since they have been cond the disclosure as filed (Rule 70.2(c)):
		(Any replacement sh report.)	eet containing such amendments must be referred to under item 1 and annexed to this
6.	Add	ditional observations, i	necessary:
111.	. Noi	n-establishment of o	pinion with regard to novelty, inventive step and industrial applicability
	The	e questions whether the	e claimed invention appears to be novel, to involve an inventive step (to be non- ally applicable have not been examined in respect of:
		the entire international	al application.
	\boxtimes	claims Nos. 2,3,6,9-1	6,18.
be	caus	se:	
		the said international not require an interna	application, or the said claims Nos. relate to the following subject matter which does tional preliminary examination (<i>specify</i>):
	⊠	the description, claims 15, 16, 18 (see separ- see separate sheet	s or drawings (<i>indicate particular elements below</i>) or said claims Nos. 2, 9,10, 12, 13, ate sheet). are so unclear that no meaningful opinion could be formed (<i>specify</i>):
		the claims, or said cla	ims Nos. are so inadequately supported by the description that no meaningful opinion
	\boxtimes	no international searc	h report has been established for the said claims Nos. 3, 6, 10, 12, 13, 16
	and/	eaningful international or amino acid sequend uctions:	preliminary examination report cannot be carried out due to the failure of the nucleotide ce listing to comply with the standard provided for in Annex C of the Administrative
		the written form has no	ot been furnished or does not comply with the standard.
			e form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA99/01157

citations and explanations supporting such statement

1. Statement

Novelty (N)

Yes:

Claims 1,4,5,7,8,17

No:

Claims

Inventive step (IS)

Yes:

Claims

Claims 1,4,5,7,8,17

No: Cla

Claims 1,4,5,7,8,17

Yes: No:

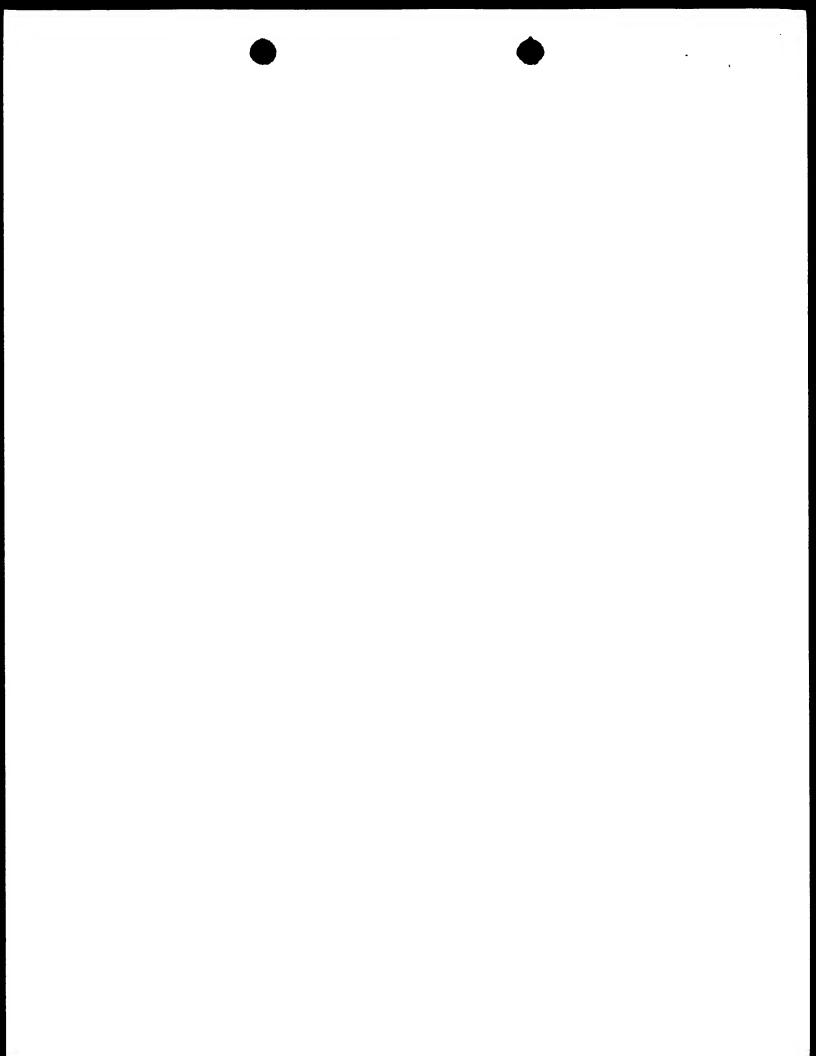
Claims

2. Citations and explanations see separate sheet

Industrial applicability (IA)

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made: see separate sheet



III.

1). There appears to be 10 from 18 independent claims. This is totally unreasonable under Rule 6 PCT. Furthermore there appears to be no basis in the original application under Article 34 PCT given for new claims 3, 6 (in cancer cells in general),12, 13, for the use of MCC without M-DNA (claims 10, 16 and 18). As these claims have not been searched and the scope of protection sough is fully unclear to the IPEA, the Examination can only therefore be based on the first independent claim in each Category, i.e. claims 1 and 17 (Article 34(4)(a) and Rule 66(1)(e) PCT).

V.

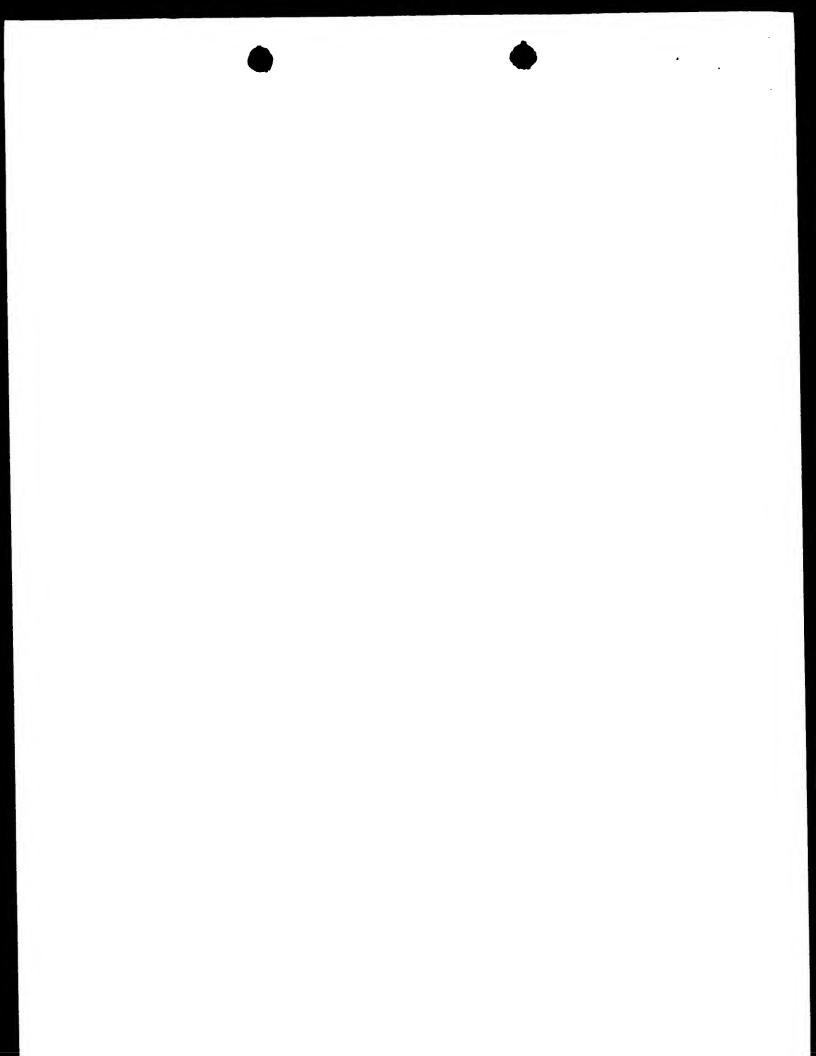
2). Document D1 (Filion et al 1998 J.Pharm.Pharmacol. 50;39, enclosed) discloses that DNA from mycobacterium phlei (M-DNA) has been shown to induce apoptosis in cancer cells.

The use of M-DNA with another chemotherapeutic agent is not disclosed, in the prior art. (Art 33(2) PCT).

However it would seem obvious to the skilled man in cancer therapy to combine two known antineoplastic agents. The subject-matter of claims 1, 4, 5, 7, 8 and 17 therefore appear not to meet the requirements of Article 33(3) PCT.

VIII.

- 3). The amendments to pages 15 and 16 do not meet the requirements of Article 34 PCT. Although there is a contradiction between the amended areas and page 16 paragraph 1, it is not immediately evident which one of the given units is in error.
- 4). For the assessment of the present claims 1 and 3-8 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.



W_

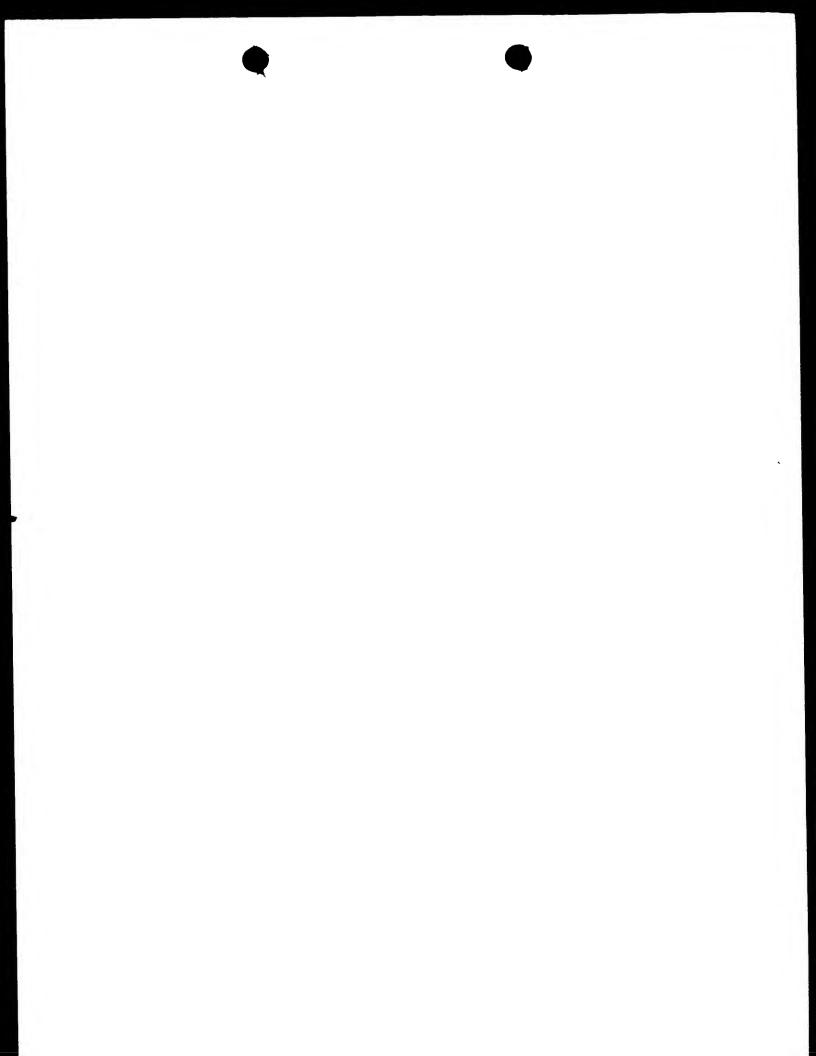


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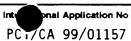
INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

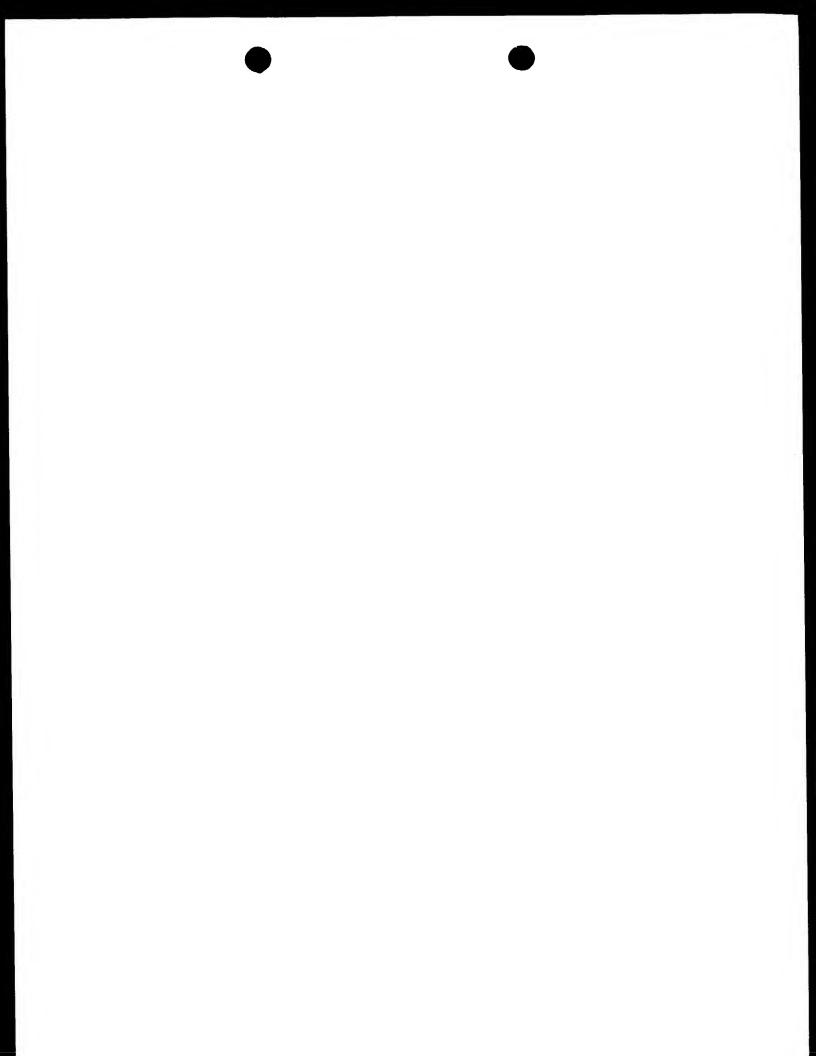
Applicant's or agent's file reference 6857-7		of Transmittal of International Search Report 220) as well as, where applicable, item 5 below.
International application No.	International filing date (day/month/year)	(Earliest) Priority Date (day/month/year)
PCT/CA 99/01157	03/12/1999	04/12/1998
Applicant		
BIONICHE INC. et al.		
This International Search Report has bee according to Article 18. A copy is being to	n prepared by this International Searching Aut ansmitted to the International Bureau.	hority and is transmitted to the applicant
This International Search Report consists It is also accompanied by	of a total of3 sheets. a copy of each prior art document cited in this	s report.
Basis of the report		
	international search was carried out on the ba less otherwise indicated under this item.	sis of the international application in the
the international search w Authority (Rule 23.1(b)).	vas carried out on the basis of a translation of	the international application furnished to this
b. With regard to any nucleotide ar was carried out on the basis of the		nternational application, the international search
contained in the internation	onal application in written form.	
	emational application in computer readable for	m.
)[this Authority in written form.	
)[this Authority in computer readble form.	
	bsequently furnished written sequence listing one is the sequence listing of t	does not go beyond the disclosure in the
the statement that the info furnished	ormation recorded in computer readable form	is identical to the written sequence listing has been
2. Certain claims were fou	nd unsearchable (See Box I).	
3. Unity of Invention is lac	king (see Box II).	
4. With regard to the title ,		
the text is approved as su	ubmitted by the applicant.	
the text has been establis	shed by this Authority to read as follows:	
5. With regard to the abstract ,		
	ubmitted by the applicant. shed, according to Rule 38.2(b), by this Author a date of mailing of this international search re	
6. The figure of the drawings to be pub	_	
as suggested by the appl		None of the figures.
because the applicant fail		
	characterizes the invention.	



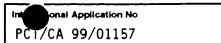
INTERNATIONAL SEARCH REPORT



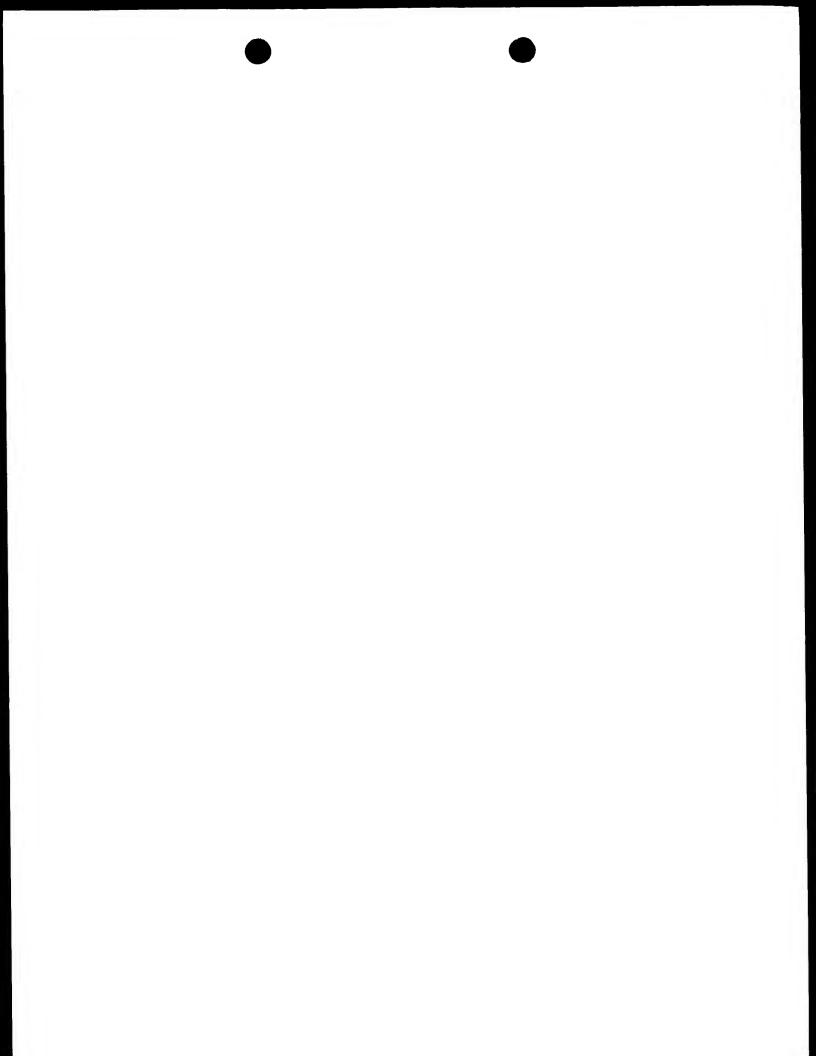
A. CLASSIFICATION OF SUBJECT MATTER IPC 7 A61K45/06 A61F A61P31/00 According to International Patent Classification (IPC) or to both national classification and IPC **B. FIELDS SEARCHED** Minimum documentation searched (classification system followed by classification symbols) IPC 7 A61K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) C. DOCUMENTS CONSIDERED TO BE RELEVANT Category * Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. S.YAMAMOTO E.A.: "In vitro augmentation 1 Α of natural killer cell activity and production of interferon alpha/beta and -gamma with deoxyribonucleic acid fraction from mycobacterium bovis BcG" JAPANESE JOURNAL OF CANCER RESEARCH, vol. 79, 1988, pages 866-873, XP002085535 page 866 page 872, column 1 -/--X Patent family members are listed in annex. X Further documents are listed in the continuation of box C. Special categories of cited documents: "T" later document published after the international filing date or priority date and not in conflict with the application but "A" document defining the general state of the art which is not considered to be of particular relevance cited to understand the principle or theory underlying the invention *E* earlier document but published on or after the international *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention citation or other special reason (as specified) cannot be considered to involve an inventive step when the document is combined with one or more other such docu-"O" document referring to an oral disclosure, use, exhibition or ments, such combination being obvious to a person skilled in the art. "P" document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family Date of the actual completion of the international search Date of mailing of the international search report 12 April 2000 18/04/2000 Authorized officer Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016 Peeters, J



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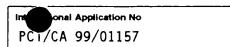


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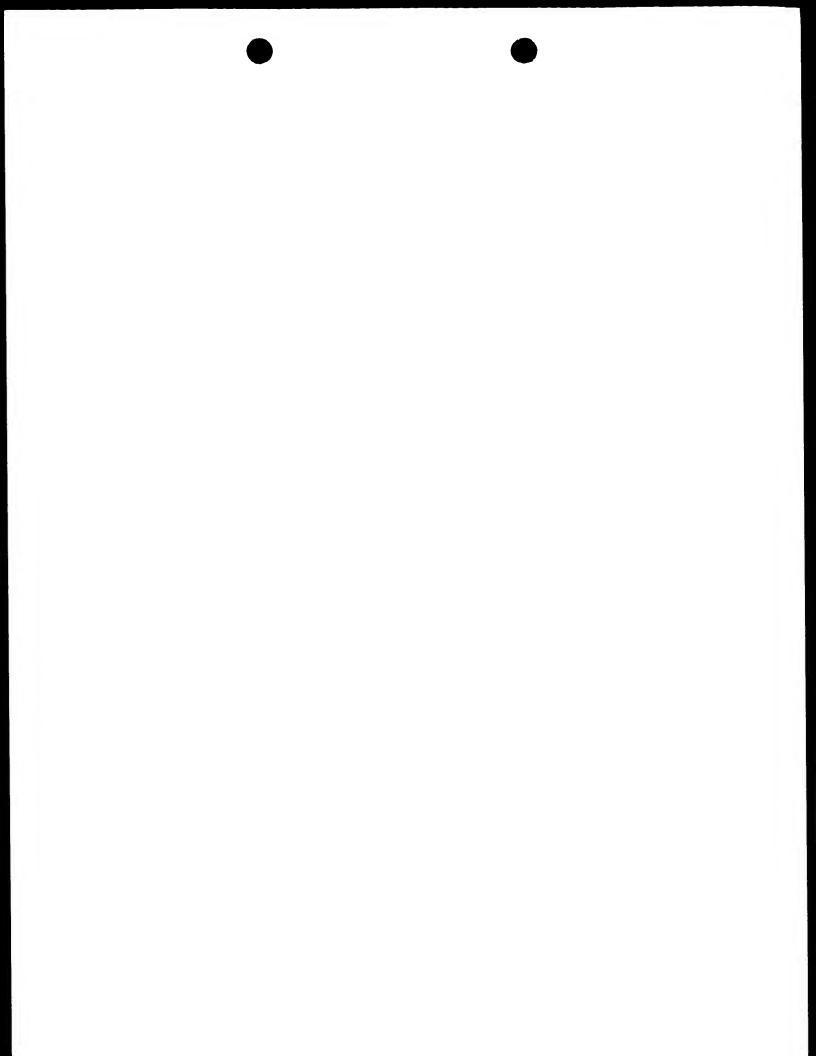


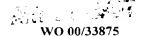
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Mitomycin-C is an anti-tumor antibiotic produced by Streptomyces caespitosus, which cross-links DNA, depolymerizes DNA and forms free radicals.

Figure 2 shows that, with B-16 cells, 0.1 μ g/ml mitomycin-C inhibited proliferation about 5%, 1 μ g/ml about 10%, and 10 and 100 μ g/ml 100%, whereas 1 μ g/ml MCC inhibited proliferation about 25%, 10 μ g/ml about 50% and 100 μ g/ml about 80%. Figure 2 also shows that, in the presence of 1 μ g/ml MCC, 0.1 μ g/ml mitomycin-C inhibited proliferation about 40%, 1 μ g/ml about 65% and 100 μ g/ml 100%. These data show that MCC potentiates the antineoplastic effect of mitomycin-C on proliferating cancer cells.

B-16 melanoma cells were incubated with 0.01 to 100 μ g/ml of 5-fluorouracil, with 1 to 100 μ g/ml of MCC and with 0.01 to 10 μ g/ml of 5-fluorouracil + 1 μ g/ml MCC. 5-fluorouracil is an antimetabolite, which interferes with DNA and RNA synthesis.

Figure 3 shows that, with B-16 cells, $0.01\mu g/ml$ 5-fluorouracil inhibited proliferation about 8%, $0.1\,\mu g/ml$ about 50%, $1\,\mu g/ml$ about 90%, and 10 and 100 $\mu g/ml$ 100%, whereas $1\,\mu g/ml$ MCC inhibited proliferation about 25%, $10\,\mu g/ml$ about 50% and 100 $\mu g/ml$ about 80%. Figure 3 also shows that, in the presence of $1\,\mu g/ml$ MCC, $0.01\,\mu g/ml$ 5-fluorouracil inhibited proliferation about 75%, $0.1\,\mu g/ml$ about 85%, $1\,\mu g/ml$ about 90% and $10\,\mu g/ml$ 100%. These data show MCC potentiates the antineoplastic effect of 5-fluorouracil on proliferating cancer cells.

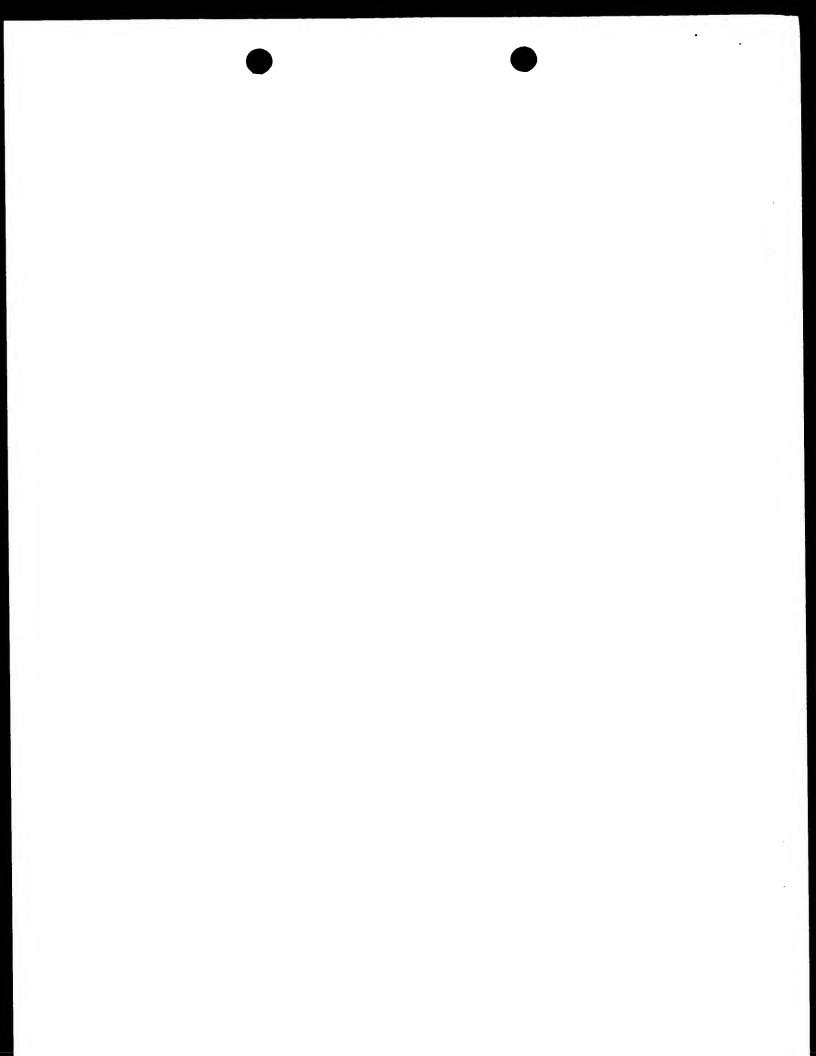
B-16 melanoma cells were incubated with 0.01 to 100 μ g/ml of cisplatin, with 1 to 100 μ g/ml of MCC and with 0.01 to 10 μ g/ml of cisplatin + 1 μ g/ml MCC. Cisplatin is an alkylating agent that cross-links DNA and inhibits DNA precursors.

Figure 4 shows that, with B-16 cells, 0.01 μ g/ml cisplatin inhibited proliferation 0%, 0.1 μ g/ml about 8%, 1 μ g/ml about 62%, 10 μ g/ml about 90% and 100 μ g/ml 100%, whereas 1 μ g/ml MCC inhibited proliferation about 25%, 10 μ g/ml about 50% and 100 μ g/ml about 80%. Figure 4 also shows that, in the presence of 1 μ g/ml MCC, 0.01 μ g/ml cisplatin inhibited proliferation about 40%, 0.1 μ g/ml about 50%, 1 μ g/ml about 70% and 10 μ g/ml about 90%. These data show that MCC enhances the antineoplastic effect of cisplatin on proliferating cancer cells.

Table 5 shows the concentrations of mitomycin-C, 5-fluorouracil, and cisplatin required for 50% inhibition of B-16 melanoma cell division in the absence and in the presence of $1\,\mu\text{g/ml}$ MCC.

Table 5

Concentration of mitomycin-C, 5-fluorouracil and cisplatin required for 50% inhibition of B-16 melanoma cell proliferation in the absence and in the presence of 1 mg/ml MCC



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T	IC ₅₀ *, μg/ml		
Treatment	Drug Alone	Drug + MCC at 1 mg/ml	
MCC	10	Not applicable	
Cisplatin	0.6	0.16	
5-Fluorouracil	0.12	0.005	
Mitomycin-C	2.2	0.12	

*concentration for 50% inhibition

Table 5 shows the dose dependent inhibition of B-16 cell melanoma proliferation by MCC at 10 to 100 μ g/ml (IC₅₀=10 μ g/ml) and by mitomycin-C, 5fluorouracil and cisplatin at 0.1 to 10 μ g/ml (IC₅₀=2.2, 0.12 and 0.6 μ g/ml respectively). Table 5 also shows that $1 \mu g/ml$ MCC potentiated mitomycin-C (IC₅₀=0.12 $\mu g/ml$) and 5-fluorouracil (IC $_{50}$ =0.005 $\mu g/ml$) inhibition of B-16 melanoma cell proliferation and that 1 µg/ml MCC enhanced cisplatin (IC₅₀=0.16 µg g/ml) inhibition of B-16 melanoma proliferation

These data show that MCC not only inhibits cancer cell proliferation, but also potentiates the antineoplastic effects of mitomycin-C and 5-fluorouracil on cancer cell proliferation and enhances the antineoplastic effect of cisplatin on cancer cell proliferation.

EXAMPLE 12

Induction of apoptosis in B-16 melanoma cells by MCC and M-DNA

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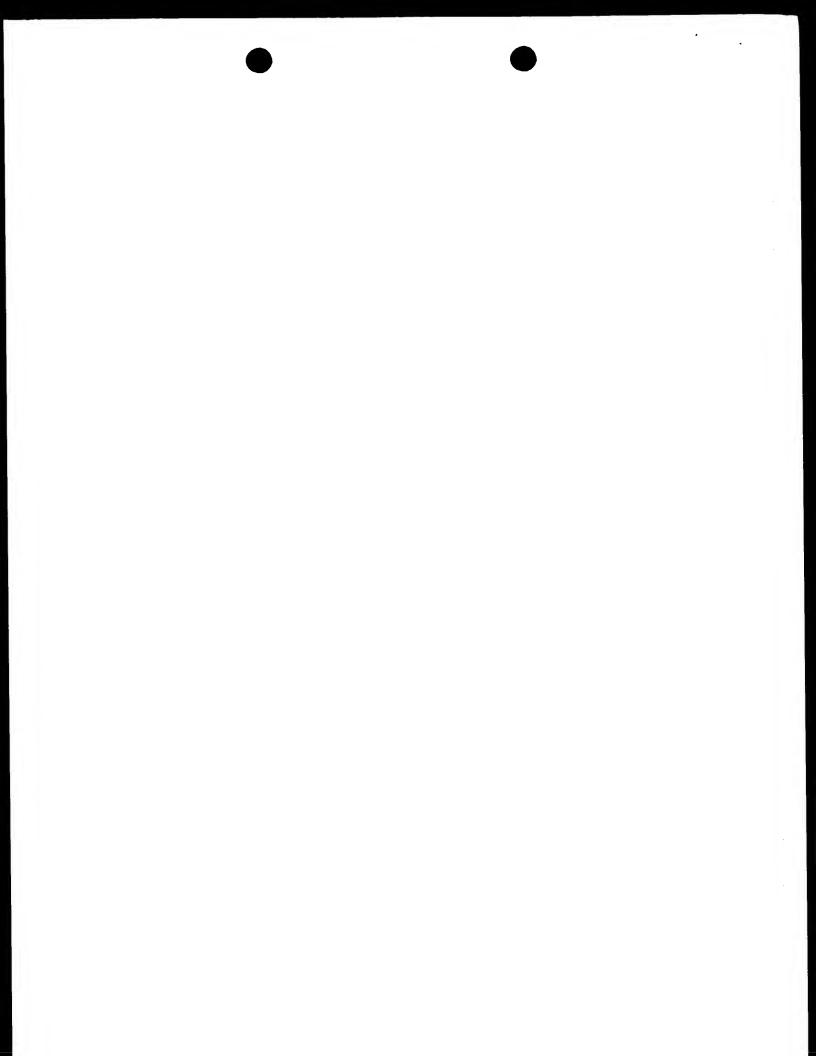
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Fragmentation of cellular DNA into nucleosome-sized fragments is characteristic of cells undergoing apoptosis (Newell et al. Nature 357:286-289, 1990). To assess DNA fragmentation, B-16 cells were lysed with 0.5 ml of hypotonic lysing buffer (10 mM Tris buffer, 1 mM EDTA, 0.2% t-octylphenoxypolyethoxyethanol (Triton X-100), pH 7.5). The lysates were centrifuged at 13,000 g for 10 min and the supernatants, containing fragmented DNA, were precipitated overnight at -20°C in 50% isopropanol and 0.5 M NaCl. The precipitates were collected by centrifugation and were analyzed by electrophoresis in 0.7% agarose gels for 3 h at 100V.

B-16 melanoma cells, at 3 X 10⁵ cells/ml, were incubated for 72 h with 1 µg/ml M-DNA (Figure 6, lane 1) and with 100 (lane 2), 10 (lane 3) and 1 μ g/ml MCC (lane 4). M-DNA and MCC treated B-16 melanoma cells showed significant DNA fragmentation, whereas untreated B-16 melanoma cells (Figure 6, lane 5) showed no DNA fragmentation. A 123-bp DNA ladder (Gibco Life Science) was used to determine the molecular weight of the nucleosome-sized DNA fragments (Figure 6, lane L). These data show that M-DNA and MCC induce apoptosis in B-16 melanoma cells.



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We claim:

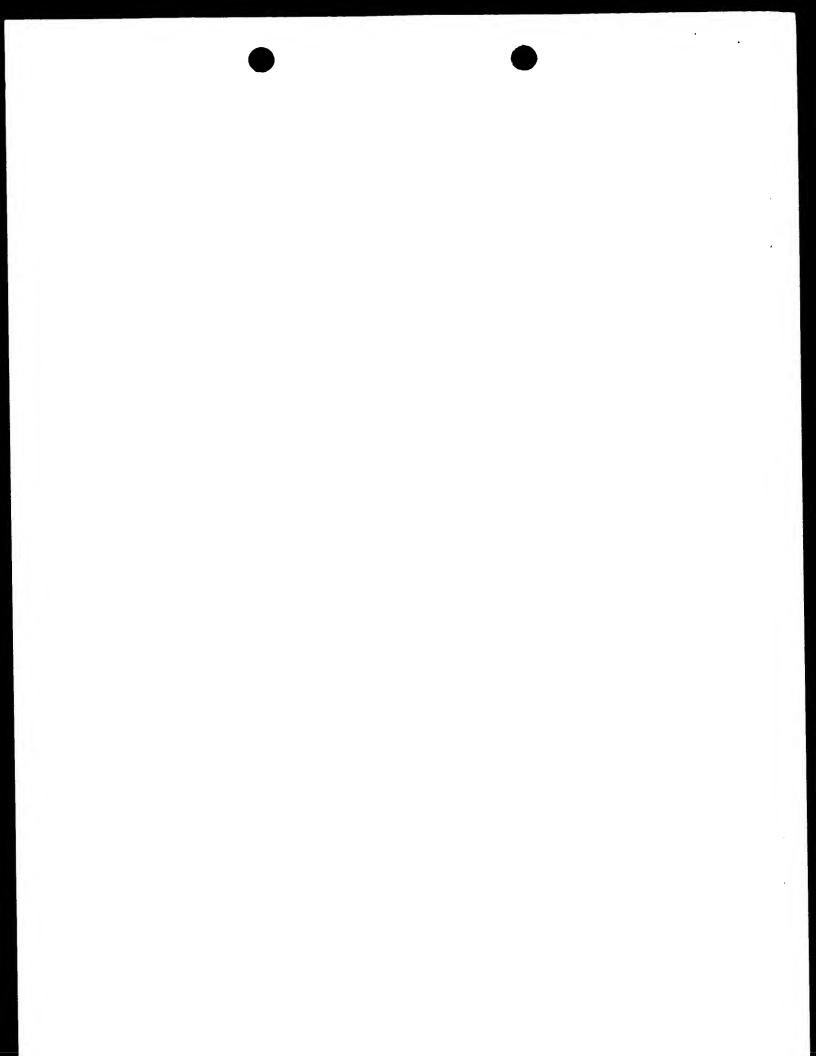
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1. A composition, comprising M-DNA, a chemotherapeutic agent and a pharmaceutically acceptable carrier, wherein the M-DNA potentiates the antineoplastic effect of the chemotherapeutic agent on cancer cells.

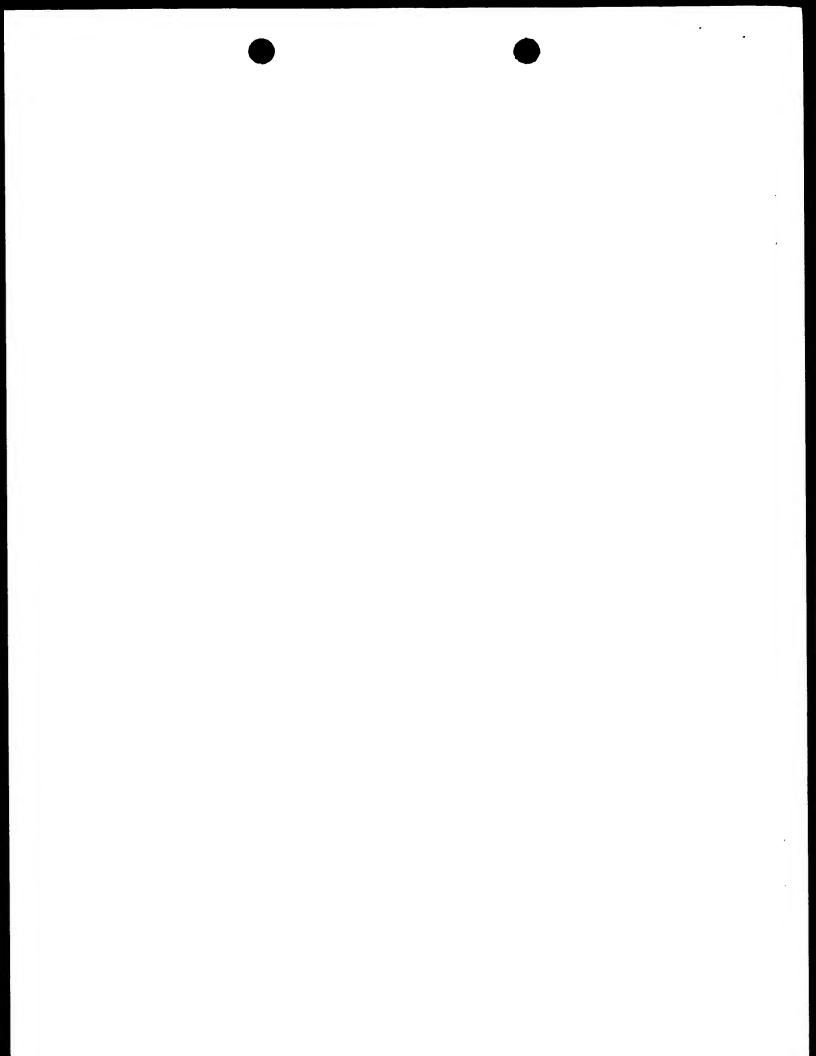
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- 5 2. A composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC), a chemotherapeutic agent and a pharmaceutically acceptable carrier, wherein the MCC potentiates the antineoplastic effect of the chemotherapeutic agent on cancer cells.
- 3. The composition of claims 1 or 2, wherein the antineoplastic effect is inhibition of proliferation of the cancer cells.
 - 4. The composition of claims 1, 2 or 3 wherein the cancer is selected from the group consisting of leukemia, lymphoma and melanoma.
 - 5. The composition of claim 4, wherein the cancer is melanoma.
- 6. The composition of claims 1, 2, 3, 4 or 5 wherein the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous carrier and a non-aqueous carrier.
 - 7. A method, comprising administering to an animal having cancer a composition comprising M-DNA, a chemotherapeutic agent and a pharmaceutically acceptable carrier, wherein the amount of the M-DNA administered to the animal is effective to potentiate the antineoplastic effect of the chemotherapeutic agent on cancer cells in the animal having the cancer.
 - 8. A method, comprising administering to an animal having cancer a composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC), a chemotherapeutic agent and a pharmaceutically acceptable carrier, wherein the amount of the MCC administered to the animal is effective to potentiate the antineoplastic effect of the chemotherapeutic agent on cancer cells in the animal having the cancer.
 - 9. The method of claims 7 or 8, wherein the antineoplastic effect is inhibition of proliferation of the cancer cells.



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- 10. A method, wherein a composition comprising M-DNA and a pharmaceutically acceptable carrier is administered to an animal having cancer in an amount effective to induce cell cycle arrest in cancer cells in the animal having the cancer.
- 11. A method, wherein a composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC) and a pharmaceutically acceptable carrier is administered to an animal having cancer in an amount effective to induce cell cycle arrest in cancer cells in the animal having the cancer.
 - 12. The method of claims 10 or 11, wherein the cell cycle arrest is induced at phase SL+GM2 of the cell cycle.
- 13. A method, wherein a composition comprising M-DNA and a pharmaceutically acceptable carrier is administered to an animal having cancer in an amount effective to induce synchronization of cancer cells in the animal having the cancer.
- 14. A method, wherein a composition comprising M-DNA preserved and complexed on M. phlei cell wall (MCC) and a pharmaceutically acceptable carrier is administered to an animal having cancer in an amount effective to induce synchronization of cancer cells in the animal having the cancer.
 - 15. A method according to claim 10, 11, 12, 13 or 14, wherein the cancer is selected from the group consisting of leukemia, lymphoma and melanoma.
 - 16. A method according to claim 15, wherein the cancer is melanoma.
- 20 17. A method according to claim 10, 11, 12, 13 or 14, wherein the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous carrier and a non-aqueous carrier.
 - 18. A method, wherein a composition comprising M-DNA and a pharmaceutically acceptable carrier is administered to an animal having melanoma in an amount effective to treat the melanoma in the animal.
 - 19. The method of claim 18, wherein the M-DNA induces apoptosis in melanoma cells of the melanoma.



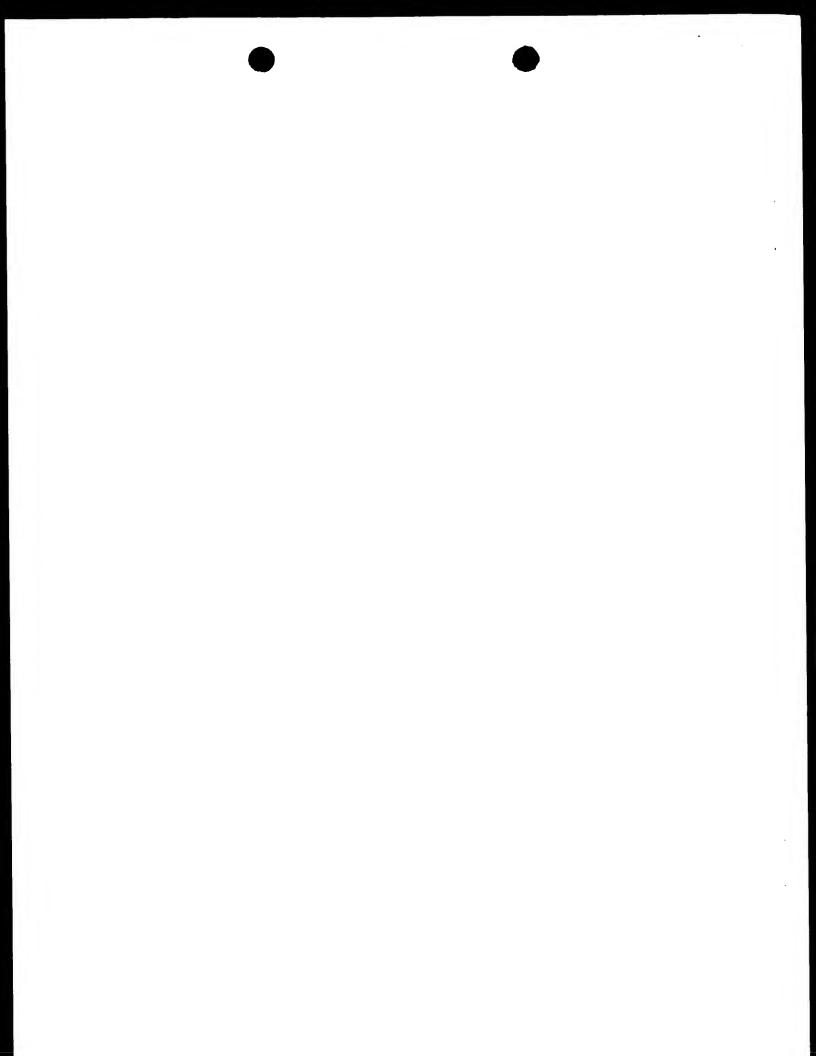
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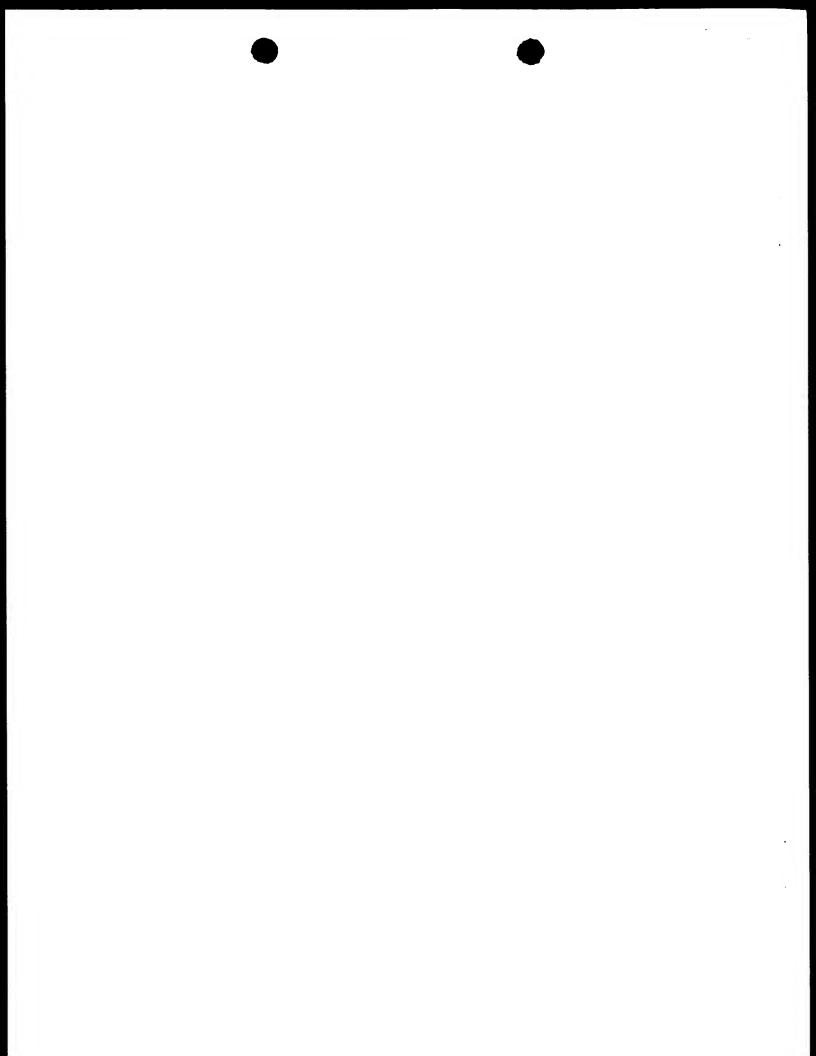
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- 20. The method of claim 18, wherein the M-DNA inhibits proliferation of melanoma cells in the melanoma.
- 21. A method, wherein a composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC) and a pharmaceutically acceptable carrier is administered to an animal having melanoma in an amount effective to treat the melanoma in the animal.
 - 22. The method of claim 21, wherein the MCC induces apoptosis in melanoma cells of the melanoma.
- 23. The method of claim 21, wherein the MCC inhibits proliferation in melanoma cells of the melanoma.
 - 24. A method according to claim 18 or 22, wherein the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous carrier and a non-aqueous carrier.
- 25. A use of a composition comprising M-DNA, a chemotherapeutic agent and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having cancer in an amount effective to potentiate the antineoplastic effect of the chemotherapeutic agent on cancer cells in the animal having the cancer.
- 26. A use of a composition comprising M-DNA preserved and complexed on M. phlei cell wall (MCC), a chemotherapeutic agent and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having cancer wherein the amount of the MCC administered to the animal is effective to potentiate the antineoplastic effect of the chemotherapeutic agent on cancer cells in the animal having the cancer.
- 25 27. The use of claim 25 or 26, wherein the antineoplastic effect is inhibition of proliferation of the cancer cells.
 - 28. A use of a composition comprising M-DNA and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having cancer in an amount effective to induce cell cycle arrest in cancer cells in the animal having the cancer.



- 29. A use of a composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC) and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having cancer in an amount effective to induce cell cycle arrest in cancer cells in the animal having the cancer.
- 5 30. A use according to claim 28 or 29, wherein the cell cycle arrest is induced at phase SL+GM2 of the cell cycle.
 - 31. A use of a composition comprising M-DNA and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having cancer in an amount effective to induce synchronization of cancer cells in the animal having the cancer.
 - 32. A use of a composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC) and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having cancer in an amount effective to induce synchronization of cancer cells in the animal having the cancer.
- 15 33. A use according to claim 28, 29, 30, 31 or 32, wherein the cancer is selected from the group consisting of leukemia, lymphoma and melanoma.
 - 34. A use according to claim 33, wherein the cancer is melanoma.
- 35. A use according to claim 28, 29, 30, 31 or 32, wherein the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous carrier and a non-aqueous carrier.
 - 36. A use of a composition comprising M-DNA and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having melanoma in an amount effective to treat the melanoma in the animal.
- 37. A use according to claim 36, wherein the M-DNA induces apoptosis in melanoma cells of the melanoma.
 - 38. A use according to claim 36, wherein the M-DNA inhibits proliferation of melanoma cells in the melanoma.



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- 39. A use of a composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC) and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having melanoma in an amount effective to treat the melanoma in the animal.
- 5 40. A use according to claim 39, wherein the MCC induces apoptosis in melanoma cells of the melanoma.
 - 41. A use according to claim 39, wherein the MCC inhibits proliferation in melanoma cells of the melanoma.
- 42. A use according to claim 36 or 40, wherein the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous carrier and a non-aqueous carrier.

